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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A23C 9/12

A1

(11) International Publication Number: WO 00/05971

(43) International Publication Date: 10 February 2000 (10.02.00)

(21) International Application Number: PCT/US99/16870

(22) International Filing Date: 27 July 1999 (27.07.99)

(30) Priority Data:
60/094,269 27 July 1998 (27.07.98) US

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(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: METHODS OF WHITENING MILK PRODUCTS

(57) Abstract

The present invention relates to novel methods for the visual color enhancement of milk products. In one method, the pH of the milk product is reduced using an acid, such as lactic acid, to increase whiteness. In another method, biological particles, such as bacterial cells, are added to a milk product to increase whiteness.

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Methods of Whitening Milk Products

Background of the Invention

Field of the Invention

This invention relates to whitening agents and methods for the manufacture of a milk product with an improved appearance.

Related Art

Milk consumption in the United States has become an integral aspect in human nutrition. Milk products provide several nutrients to living organisms for their continued health and growth. Unfortunately, many milk products also contain high amounts of fat. With current research linking the consumption of large quantities of fat to health problems in later life, the public has requested healthier alternatives to milk products containing large amounts of milk fat.

In milk production, an important process step involves the separation of milk to yield skim milk, i.e., non-fat milk, and cream. Skim milk has a very low fat content, less than about 0.5%, and is considered the healthiest of all milk products. By mixing non-fat milk and cream, milk may be standardized to a desired fat content to produce milk products commonly known as 1%, 2%, and whole milk (3.25%).

Due to an increased awareness of the health problems associated with fat-containing milk products, per capita sales of whole milk and 2% milk have decreased significantly, while sales of non-fat milk products have steadily increased over the last five years. However, the modest increase in non-fat milk product sales has not compensated for the large decrease in per capita sales of low-fat and whole milk products (Milk Industry Foundation, 1995). Despite several aggressive marketing efforts to increase non-fat milk product sales and exploit its healthful benefits, the appearance and texture of non-fat milk, with its blue-gray color and watery mouthfeel, are viewed as hurdles towards the general public's acceptance of non-fat milk products (Tripician, 1995). Consumers prefer

milk that has the visual characteristics, i.e., the white color, of whole milk (Tuorila, 1987).

Perceptions of non-fat milk's appearance often affects perceptions of its taste and mouthfeel, though the appearance remains a primary factor of objection. An investigation of the properties of milk affecting consumers' sensory responses revealed that increased visual "whiteness" has the most positive influence on increasing consumer appeal (Phillips *et al.*, 1995). Moreover, studies have shown that when color is improved, one's perceptions of non-fat milk's flavor and mouthfeel also improve.

Milk whiteness is caused by suspended particles in milk fat globules that scatter light in the visible spectrum. Since the majority of the milk fat globules are removed in non-fat milk, the number, distribution and size of the suspended particles are reduced which diminishes light-scattering. This reduction in light scattering gives non-fat milk its blue-gray color.

Several technologies designed to enhance the visual appearance of non-fat milk have been investigated and have been partially successful (Savello, 1994; Phillips *et al.*, 1997; Quiñones *et al.*, 1996; Wahid-ul-Hamid and Manus, 1960). One study involved the reaction of rennet enzymes with casein micelles as a coagulant to change the light-refractive properties of milk protein (Jeffries and Ogden, 1996). The inclusion of food-grade whitening agents, such as titanium dioxide, which directly increase perceived whiteness as a function of concentration, is another method. Additionally, several commercial additives are available which rely on a blend of food-grade colorants and stabilizing agents, such as carboxymethyl cellulose, to improve the whiteness and viscosity of non-fat milk products (Jeffries and Ogden, 1996; Tripician, 1995). However, since many of these additives are perceived as unnatural by consumers or are inorganic compounds, many consumers find them objectionable. Moreover, many of the current additives are not cost effective due to high production costs. These limitations have negatively impacted sales of the additives and most likely have stymied further marketing efforts (Light *et al.*, 1992).

Summary of the Invention

This invention seeks to implement alternative technology to provide a milk product with improved visual appearance.

This invention relates to a composition for whitening a milk product comprising a milk product and at least one biological particle that has an average diameter of between about 200-2500 nm in an amount effective to whiten the milk product.

This invention further relates to a method for whitening a milk product comprising adding to a milk product an effective amount of at least one biological particle which has an average diameter of about 200-2500 nm.

The invention further relates to a method for whitening a milk product comprising reducing the pH of a milk product to between about 4.0 to 4.8.

Brief Description of the Figures

Figs. 1A-1C are bar graphs illustrating the "L," "a," and "b" values of acidified non-fat milk products (pH 4.0, 4.6, 5.0, and 6.0), non-treated non-fat milk (NM), and 2% reduced fat milk (TM).

Fig. 2 is a graph illustrating the "L" and "b" values over the visible spectrum for acidified milk products (pH 4.0, 4.6, 5.0, and 6.0), non-treated non-fat milk (NM), 2% reduced fat milk (TM), *Propionibacterium freudenreichii* ssp *shermanii*-containing non-fat milk (PM), and *Lactococcus lactis* ssp *lactis*-containing non-fat milk (LM).

Figs. 3A-3F are bar graphs illustrating the percent reflectance and transmission values for NM, HBM, and TM milk products (NM = non-treated non-fat milk, TM = 2% reduced fat milk, and HBM = high bacterial cell population-containing non-fat milk).

Fig. 4 is a graph illustrating percent reflectance for *Propionibacterium freudenreichii* ssp *shermanii*-containing non-fat milk (PM), *Lactococcus lactis* ssp

lactis-containing non-fat milk (LM), non-treated non-fat milk (NM), and 2% reduced fat milk (TM).

Fig. 5 is a graph illustrating percent transmission for milk with added bacterial cultures (*Lactococcus lactis* ssp *lactis*-containing non-fat milk (LM), and *Propionibacterium freudenreichii* ssp *shermanii*-containing non-fat milk (PM)), non-treated non-fat milk (NM), and 2% reduced fat milk (TM).

Fig. 6 is a graph illustrating percent reflectance for acidified milk (pH 4.0, 4.6, 5.0, and 6.0), non-treated non-fat milk (NM), and 2% reduced fat milk (TM).

Fig. 7 is a graph illustrating percent transmission for acidified milk (pH 4.0, 4.6, 5.0, and 6.0), non-treated non-fat milk (NM), and 2% reduced fat milk (TM).

Detailed Description of the Preferred Embodiments

Milk products can be treated using at least one of the inventive methods to enhance the visual appearance. The final milk product has a white color similar with reflectance and transmission values to a milk product with a higher fat content. In preferred embodiments, the milk product is a non-fat milk product.

Desired whiteness can be measured, *inter alia*, by ΔE values which are explained below. A desirable ΔE value falls below about 5.50 as measured by either a GE Cool White lamp or a tungsten filament lamp.

In one method, the pH of a milk product is reduced by the addition of an acid to increase average particle size to improve the color of milk products. In a preferred embodiment, the milk product is treated with an organic and/or inorganic acid such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulphuric acid, carbonic acid, citric acid, lactic acid, tartaric acid, malic acid, fumaric acid, mandelic acid, or oxalic acid to reduce the pH of the milk product from about 6.5 (native pH of milk) to about 4.0-6.0. In a more preferred embodiment, the acid is an organic acid. In a most preferred embodiment, the organic acid is lactic acid. The pH is preferably reduced to between about 4.0 and 4.8. In a more preferred embodiment, the pH is reduced to about 4.0-4.6. In a most preferred embodiment, the pH is reduced to about 4.6.

In another method, the visual appearance of milk products is improved by the addition of at least one biological particle thereto. The biological particles function as light scattering suspended particles to increase the whiteness of the milk product. In a preferred embodiment, the biological particles are bacterial cells. In a more preferred embodiment, the bacterial cells have an average diameter of between about 200-2500 nm, exhibit little or no growth in milk, and/or are obtained from a starter culture. In a more preferred embodiment, the particle size is between about 600-900 nm or between about 1000 and 2500 nm. In another preferred embodiment, the particle size is between about 200-350 nm since optimal light diffraction is achieved when the particles have a diameter corresponding to approximately half the wavelength of light (approximately 400-700 nm). However, any size biologically-produced particle with the physiological and metabolic features required to function as a light-scattering particle to diffract light within the visible spectrum in a fluid may be employed in the present invention. Preferably, the biological particles are non-pathogenic. Examples include bacterial cells and spores and virus particles.

Bacteria effective in this method include lactic acid bacteria such as *Propionibacterium freudenreichii* ssp *shermanii* (PFS) (average diameter size of about 700-800 nm) and *Lactococcus lactis* ssp *lactis* (average diameter size of about 1000-2500 nm). PFS is preferred due to its smaller size. PFS is recognized in the dairy industry for its use in Swiss cheese, where it contributes to flavor development and eye formation. PFS has low proteolytic activity, does not ferment lactose, and remains in a vegetative state in milk. Small size, low protease presence, and slow growth in milk are desirable characteristics for biological milk whiteners.

The desired biological particles can be added to a milk product directly. If the biological particles are bacterial cells, then the bacteria are first cultured, using conventional culture techniques, and harvested to produce bacterial cell pellets which are then suspended into the milk product.

The phrases "milk products" and "milk product" encompass low-fat milk, 1% milk, 2% milk, whole milk, non-fat milk, and other varieties of milk including

flavored milk and specific dietary milk, such as Lactaid®, as well as other milk-containing or milk fat-containing products such as cream, ice cream, cheese, cottage cheese, sour cream, yogurt, etc. In addition, the methods of the invention can be combined and/or used in combination with known methods, such as the addition of a whitening agent such as titanium dioxide.

The methods of the invention may be used in a wide variety of food applications where whiteness is a desired attribute. The invention could also be used to produce a healthier milk product since the bacteria that may be added to the milk product could have a probiotic effect.

Having now generally described this invention, the same will be understood by reference to the following examples which are provided herein for purposes of illustration only and are not intended to be limiting unless otherwise specified.

Examples

Example 1 - Effects of Fermentation on the Whiteness of Non-fat Milk

Bacterial Cultures and Fermentation Conditions

Strains of *Lactococcus lactis* ssp *lactis* (SCO 230) were obtained from Chris Hansen's Laboratory, Milwaukee, WI. Culture preparations were initially cultivated in sterile 11% reconstituted non-fat milk, containing 11 grams of dry milk brought to a final volume of 100 ml, for about 24 hours at about 25°C. Cell pellets were washed by suspension in peptone buffer and centrifuged to obtain a final cell mass of approximately 5 g. Pellets were suspended in non-fat milk to achieve a final bacterial cell concentration of approximately 10^9 CFU/ml.

Colorimeter Measurements

After cultivation and harvestation, statistically significant differences between the color of fermented non-fat milk and non-treated non-fat milk were

measured using a spectrophotometer with light sources represented by a GE Cool White lamp (CWF) and a tungsten filament lamp (A). These illuminants mimic typical lighting sources used by consumers. The spectrophotometer was used to determine whiteness ("L"), red/greenness ("a"), and yellow/blueness ("b") values in milk. Specifically, colorimeter values of non-fat milk fermented with exopolysaccharide-producing strains of *Lactococcus lactis* ssp *lactis* (EFM) and non-exopolysaccharide-producing strains of *Lactococcus lactis* ssp *lactis* (FM) were compared to the colorimeter values of non-treated non-fat milk (NM), heat treated non-fat milk (HTC), and 2% reduced fat milk (TM). Whole milk (WM) was used as a control.

Results

Table 1 shows the colorimeter values obtained.

Table 1. Effects of Fermentation Treatments on Instrumental Measurements of Color

	Instrumental Value-Illuminant							
Treatment	L-CWF	L-A	a-CWF	a-A	b-CWF	b-A	ΔE-CWF	ΔE-A
NM	82.7^{a} (0.02)	82.4^{a} (0.02)	-4.13^{b} (0.00)	-4.72^{b} (0.00)	5.82^{a} (0.01)	4.50^{a} (0.01)	6.95^{a} (0.03)	7.65^{a} (0.02)
HTC	82.7^{a} (0.04)	82.4^{a} (0.04)	-4.56^{a} (0.00)	-5.45^{a} (0.00)	5.25^{a} (0.01)	3.94^{a} (0.02)	7.25^{a} (0.04)	8.20^{a} (0.04)
FM	87.8^{b} (0.55)	87.6^{b} (0.58)	-2.89^{c} (0.17)	-2.95^{c} (0.20)	5.71^{a} (1.46)	4.62^{a} (0.76)	2.82^{b} (1.43)	3.22^{b} (1.41)
EFM	87.6^{b} (0.26)	87.4^{b} (0.26)	-2.75^{cd} (0.05)	-2.90^{c} (0.21)	4.92^{a} (0.76)	3.81^{a} (0.77)	3.51^{b} (0.74)	3.90^{b} (0.76)
TM	88.0^{b} (0.00)	87.9^{b} (0.00)	-2.56^{d} (0.04)	-1.99^{d} (0.00)	8.21^{b} (0.01)	7.19^{b} (0.01)	1.08^{c} (0.00)	1.09^{c} (0.01)
WM	89.0^{c} (0.02)	88.9^{c} (0.01)	-2.27^{e} (0.00)	-1.65^{e} (0.00)	8.09 (0.01)	7.18^{b} (0.01)	control	control

^{a,b,c,d,e} Means in a column with different superscript letters are different ($P \leq 0.05$)

Values in parentheses denote standard deviation (n=3)

Whiteness values ("L") measured with the CWF light source ranged from a low of 82.7 for the NM sample to a high of 89.0 for the WM sample. "L" values for the HTC sample were not significantly different from the NM sample, suggesting that the observed fermentation effects were not due to thermal treatment alone. Both the EFM and FM samples had significantly higher "L" values than the NM and HTC samples, but were not significantly different than the TM sample suggesting that the fermentation of non-fat milk products with *Lactococcus lactis* ssp *lactis* can result in a substantial increase in whiteness as determined by instrumental measurement. There was no significant difference between the FM and EFM samples which suggests that the production of microbial exopolysaccharide has no effect on "L" value.

The HTC sample had a substantially lower "a" value than the NM control, possibly due to the denaturation of heat-labile proteins capable of interacting with light in the "a" region. Both the EFM and FM samples had substantially higher "a" values than the NM and HTC samples. The TM and WM samples exhibited the highest "a" values. Although there is a demonstrable influence from fermentation, "a" values are thought to have little influence on perceived whiteness (Hunter, 1975) and thus may have little significance with respect to increased whiteness.

Neither the FM nor EFM samples had any detectable effect on "b" values in that neither were significantly different than the NM or HTC sample. ΔE values (total difference) provide a cumulative measurement of total color as determined by differences in "L," "a," and "b" values as compared to a control (Drake *et al.*, 1994) and were computed using $\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$ where whole milk was the control. Smaller ΔE values suggest that instrumental measurements of color are close to the control value whereas large ΔE values denote large color differences. The HTC and NM samples had the highest ΔE values, followed by the EFM and FM samples. Although the ΔE values for the FM and EFM samples were significantly higher than the TM sample, fermentation substantially reduced ΔE values as compared to the NM and HTC samples.

Sensory analyses were conducted with a trained panel (n=15) to characterize visual changes in experimental treatments. Panelists were trained to

evaluate the specific visual attributes of center color and edge color. Samples were rated on a fourteen-point interval scale from least white to most white. Randomly coded samples of non-fat milk and whole milk were included as reference anchors during sensory panels and assigned whiteness values of 3 and 12, respectively. Samples were randomly presented in transparent plastic cups under white fluorescent lights against a black lab benchtop. Each panelist evaluated each sample a single time at one sitting. The unappealing color of non-fat milk is especially apparent at the milk surface-container side interface where light transmission is greatest. Edge color therefore, represents a critical sensory attribute for examining color of fluid milk products. Table 2 illustrates that the HTC sample contributed no detectable increase in edge color whiteness to non-fat milk, whereas the FM and EFM samples were not significantly different than the TM sample.

Table 2. Effects of Fermentation on the Sensory Attributes of Edge and Center Color

	NM	HTC	FM	EFM	TM	WM
Edge	2.43^{a}	3.44^{a}	7.13^{b}	9.33	$8.23^{b,c}$	9.44^{c}
	(0.51)	(1.83)	(2.33)	(2.56)	(1.83)	(2.42)
Center	3.27^{a}	3.01^{a}	7.25^{b}	9.33^{c}	7.64^{b}	10.3^{c}
	(1.22)	(2.08)	(1.71)	(2.58)	(2.34)	(1.75)

^{a,b,c} Means in a row with different superscript letters are different ($P \leq 0.05$)

Values in parentheses denote standard deviation (n=15)

Moreover, the EFM sample surprisingly showed no significant difference in edge color whiteness relative to the WM samples. This demonstrates that exopolysaccharide production influences edge color beyond what is gained through fermentation alone, i.e., the FM sample. Sensory evaluation of edge color, however, is influenced more by light transmission.

Example 2 - Effects of Fermentation on the Whiteness of Non-fat Milk

Bacterial Cultures and Fermentation Conditions

Two lactic acid bacteria commonly used in dairy foods were employed for this study. The first, *Lactococcus lactis* ssp. *lactis* SCO-236 (Chris Hansen's Laboratory, Milwaukee, WI) was selected for its prevalent use in fermented milk products. The second, *Propionibacterium freudenreichii* ssp. *shermanii*, was selected for its relatively small size, slow growth in milk, and low proteolytic activity.

Propionibacteria were propagated at 32°C for 48 hrs in 4 L of filter sterilized (0.2µm) growth medium consisting of: dextrose, 15g; sodium lactate 60%, 30 ml (Fisher Scientific Inc., Pittsburgh, PA); yeast extract, 15g (Difco, Inc., Detroit MI); casein hydrolysate, 60g (Pepticase®, Quest International, Hoffman Estates, IL); and lab grade water, 3L autoclaved for 15 min at 121°C. Lactococci were propagated in 2 L sterile Elliker broth (Difco, Inc., Detroit, MI) and incubated at 32°C for 48 hrs. Cells were harvested by centrifugation at 5000 g for 10 min. at 4°C. Bacterial pellets were washed twice in 0.1% peptone buffer.

Milk Sample Preparation

Non-fat milk and 2% reduced fat pasteurized, homogenized milk were obtained from a local commercial manufacturer and stored at 4°C for no longer than 2 days until analysis. Milk samples containing high concentrations of Lactococci (LM) and Propionibacteria (PM) were prepared by suspending cell pellets into the non-fat milk with a gentle swirling motion. Milk samples were removed for immediate instrumental analysis. Bacterial counts for the milk samples were determined using Standard Methods agar (Difco, Inc., Detroit, MI). A total of four treatments were evaluated: non-fat non-treated milk (NM), Lactococci-containing non-fat milk (LM), Propionibacteria-containing non-fat milk (PM), and 2% non-treated reduced fat milk (TM).

Colorimeter Measurements

A Chroma Sensor-5 dual beam spectrophotometer (Applied Color Systems, Inc., Charlotte, NC) was used to determine the color coordinates and the spectral curves for reflectance and transmission values at 10 nm intervals from 400 to 700 nm using the GE Cool White flourescent light source. Reflectance values were obtained with a 25 mm viewing area and 10° visual field using a 50 x 50 x 50 mm optical cell (Fisher Scientific Inc., Pittsburgh, PA). Transmission values were determined using a 50 x 50 x 10 mm optical cell.

Statistical Analyses

The entire experiment was replicated three times on three consecutive weeks with three different code dated milks. The experiment was designed as a randomized block and data were analyzed using Statistical Analysis System (SAS Institute v 6.12, Cary, NC). Analyses were conducted with treatment type as the main effect and day as the block. Means were compared by least significant difference (LSD). Significant treatment effect was declared at $P \leq 0.05$ level.

High Cell Count Milk

Total aerobic plate counts were 1.4×10^4, 2.1×10^5, 7.6×10^9, and 3.7×10^9 for the TM, NM, LM, and PM samples, respectively. Average pH values were 6.65, 6.67, 6.65 and 6.61 for the TM, NM, LM, and PM samples, respectively. The pH did not vary significantly between milk treatments.

Results

Reflectance ("L" values (Table 3)) were only modestly influenced by the inclusion of bacterial cells. Interestingly, the PM samples had significantly lower "L" values than any of the other samples. Furthermore, "a" values (redness/greenness) for the PM samples were not significantly different than the

sample with the highest milk fat content (TM), suggesting that red hue shift is achievable through the inclusion of Propionibacteria.

Table 3 - Reflectance Values for Milk with Added Bacterial Cultures

	NM	TM	LM	PM
L	82.13^{b}	88.03^{a}	82.50^{b}	81.02^{c}
	(0.44)†	(0.03)	(0.28)	(0.47)
a	-4.16^{a}	-2.57^{c}	-3.60^{b}	-2.77^{c}
	(0.10)	(0.06)	(0.03)	(0.38)
b	6.26^{c}	8.49^{a}	7.29^{b}	7.08^{b}
	(0.24)	(0.16)	(0.45)	(1.00)
$^{a.b.c}$ Means in a row with different superscript letters are different ($P \leq 0.05$).				
† Values in parenthesis denote standard deviation.				

Previous work demonstrated that "a" values are highly correlated with the descriptive sensory attributes of edge and center color of milk evaluation (Rankin and Brewer, 1998). Both bacteria-treated milks had significantly higher "b" values (yellowness/blueness) than the NM control, altering instrumental measurements of milk color. A spectral display of percent reflectance is depicted in Figure 4. From approximately 400-600 nm, the PM samples have the lowest values, which suggests that the small cell size Propionibacteria have different absorption properties than the more commonly used Lactococci.

The transmission values, which may be a more sensitive measurement of visual attributes related to transmitted light, such as edge color, were unpredictably different than the reflectance values (Table 4). Transmission "L" values for the LM and PM samples were significantly lower than NM sample although not as low as the TM sample. The "a" values were dramatically high for the PM sample. whereas the LM sample was not significantly different than the TM sample and the NM sample was significantly lowest. Finally, reflectance "b" values were highest for the bacteria and lowest for the TM sample. The plot of

percent transmission (Figure 5) illustrates the ability of the bacteria to decrease the transmission of light in the visible spectrum while suspended in non-fat milk.

Table 4 - Transmission Values for Milk with Added Bacterial Cultures

	NM	TM	LM	PM
L	38.11^{a}	21.78^{c}	33.93^{b}	33.12^{c}
	$(0.96)^{\dagger}$	(0.13)	(0.99)	(2.30)
a	0.61^{c}	1.56^{b}	1.29^{b}	2.56^{a}
	(0.35)	(0.14)	(0.32)	(0.59)
b	35.56^{b}	32.94^{c}	37.95^{a}	36.43^{ab}
	(1.74)	(0.50)	(0.63)	(1.55)
$^{a.b.c}$ Means in a row with different superscript letters are different ($P \leq 0.05$).				
† Values in parenthesis denote standard deviation.				

A spatial representation of all milk treatments is shown in Figure 2 illustrating the relative positions on a lightness-yellowness diagram.

Reflectance "L" values for the TM and PM samples were significantly different from the LM and NM samples. For "a" reflectance values, the TM, PM, and NM samples were significantly different from the LM sample. For "b" reflectance values, the TM and NM samples were significantly different from the LM and PM samples.

Transmission "L" values for the TM sample was significantly different from the LM and PM samples, and the PM sample was significantly different from the NM sample. Transmission "a" values for the PM and NM samples were significantly different than the TM and LM samples. For the "b" transmission values, the TM sample was significantly different than the NM and PM samples. However, although the NM sample was not different from the PM sample, it was different than the LM sample whereas the PM sample was not different from the LM sample.

Example 3 - pH Reduction in Milk

To investigate the influence of pH depression on color, non-fat milk samples were acidified with cold 85% lactic acid to pH values representative of fermentation using a calibrated pH electrode. Experimental treatments were as follows: non-fat milk at pH 6.0, 5.5, 5.0, 4.5, and 4.0. Non-fat milk at native pH (about 6.5) (NM) and 2% reduced fat milk (TM) were included for comparison.

Reflectance "L," "a," and "b" values were significantly influenced by the reduction of pH as summarized in Tables 5 and 6. Reflectance "L" values were highest for the fat-containing samples. Milk samples at pH 4.0 and 4.6, although significantly lower than the TM sample, was significantly higher than the NM and pH 6.0 samples. pH-induced destabilization of the casein micelle may possibly contribute to the increased whiteness of acidified milk, particularly at a pH of 4.6. Non-fat milks acidified to pH 5.0 generated the significantly lowest "L" values. Values closest to the TM sample value were at or near the isoelectric point of casein (pH 4.6) and values furthest from the TM sample value were obtained at pH 5.0.

The decrease in "L," "a," and "b" values at pH 5.0 is most likely attributable to the increased solubility of colloidal calcium phosphate and concomitant dissociation and increased porosity of the casein micelle (Guince *et al.*, 1993; Walstra, 1990).

Reflectance "a" and "b" values followed a decreasing pattern similar to the "L" values with minor exceptions. Reflectance "a" and "b" values for pH 5.0 and NM milk samples were not significantly different, suggesting that these measurements are not as sensitive to pH changes as much as the "L" values.

Table 5 - Reflectance Values for Acidified Milk

	NM	TM	4.0	4.6	5.0	6.0
L	82.10^{c}	87.59^{a}	83.94^{b}	84.00^{b}	80.74^{d}	82.22^{c}
	(0.48)†	(0.45)	(0.25)	(0.15)	(0.95)	(0.64)
a	-4.06^{ab}	-2.49^{d}	-3.36^{c}	-3.40^{c}	-4.22^{a}	-4.00^{b}
	(0.13)	(0.06)	(0.08)	(0.14)	(0.14)	(0.11)
b	5.71^{d}	8.52^{a}	8.09^{b}	7.52^{c}	5.45^{d}	5.77^{d}
	(0.26)	(0.10)	(0.21)	(0.10)	(0.23)	(0.32)
^{a,b,c,d} Means in a row with different superscript letters are different ($P \leq 0.05$).						
† Values in parenthesis denote standard deviation.						

Table 6 - Transmission Values for Acidified Milk

	NM	TM	4.0	4.6	5.0	6.0
L	36.87^{b}	22.0^{d}	34.03^{c}	33.23^{c}	39.87^{a}	37.07^{b}
	(1.42)†	(0.28)	(0.69)	(1.04)	(1.11)	(1.18)
a	1.07^{b}	1.70^{a}	-0.20^{d}	0.22^{c}	0.56^{c}	1.02^{b}
	(0.25)	(0.08)	(0.10)	(0.27)	(0.15)	(0.22)
b	37.57^{a}	33.59^{d}	33.06^{d}	34.71^{c}	35.56^{b}	37.11^{a}
	(0.53)	(0.43)	(0.07)	(0.51)	(0.17)	(0.55)
^{a,b,c,d} Means in a row with different superscript letters are different ($P \leq 0.05$).						
† Values in parenthesis denote standard deviation.						

Both reflectance and transmission data (Figures 6 and 7) over the visible spectrum were in agreement with "L," "a," and "b" values reflective of pH-related changes.

Example 4 - pH Reduction in Milk and Bacterial Fermentation of Milk

Colorimeter values of acidified milk (AM) and milk with high bacterial cell population (HBM) were conducted in triplicate. The entire study was repeated

three times and analyzed with ANOVA. Fisher multiple comparisons were conducted where appropriate ($\alpha = 0.05$). Untreated non-fat milk (NM) and 2% reduced fat milk (TM) were included for comparison. Reflectance "L," "a," and "b" values for the AM samples reached maxima at pH 4.6, 4.0, and 4.0, respectively, each representing statistically significant increases relative to the NM control. "L," "a," and "b" minima were obtained at pH 5.0, with values statistically less than the NM control. The HBM samples exhibited significant increases in "L," "a," and "b" values relative to the NM control, but lower than the TM control values. Relative to the NM control, the HBM sample exhibited increases in percent reflectance from 500-700 nm and decreases in percent transmission from 400-700 nm. These results demonstrate that both pH reduction and the introduction of bacteria cell populations can increase instrumental measurements of milk whiteness. *See* Figures 1A-1C and 3A-3F.

From the foregoing description, one skilled in the art can easily ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions without undue experimentation. All patents, patent applications and publications cited herein are incorporated by reference in their entirety.

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What Is Claimed Is:

1. A method for whitening a milk product, comprising adding to said milk product an effective amount of at least one biological particle having an average diameter of about 200-2500 nm.

2. The method of claim 1, wherein the particle is a non-pathogenic bacterium, virus, or spore.

3. The method of claim 2, wherein the particle is a bacterium.

4. The method of claim 3, wherein the bacterium has a particle size of about 600-900 nm.

5. The method of claim 3, wherein the bacterium is *Propionibacterium freudenreichii* ssp *shermanii* or *Lactococcus lactis* ssp *lactis*.

6. The method of claim 1, wherein the milk product is non-fat milk.

7. The method of claim 1, wherein the pH of the milk product is between about 4.0 and 4.8.

8. The method of claim 7, wherein the pH of the milk product is about 4.6.

9. A composition for whitening a milk product, comprising the milk product and at least one biological particle having an average diameter of between about 200-2500 nm in an amount effective to whiten said milk product.

10. The composition of claim 9, further comprising titanium dioxide.

11. The composition of claim 9, wherein the particle is a non-pathogenic bacterium, virus, or spore.

12. The composition of claim 11, wherein the particle is a bacterium.

13. The composition of claim 12, wherein the bacterium has a particle size of about 600-900 nm.

14. The composition of claim 12, wherein the bacterium is *Propionibacterium freudenreichii* ssp *shermanii* or *Lactococcus lactis* ssp *lactis*.

15. A method for whitening a milk product, comprising reducing the pH of the milk product to between about 4.0 to 4.8.

16. The method of claim 15, wherein the pH is reduced by adding lactic acid to the milk product.

17. The method of claim 15, wherein the pH is reduced to between about 4.0 and 4.6.

18. The method of claim 17, wherein the pH is reduced to about 4.6.

19. The method of claim 15, wherein the milk product is non-fat milk.

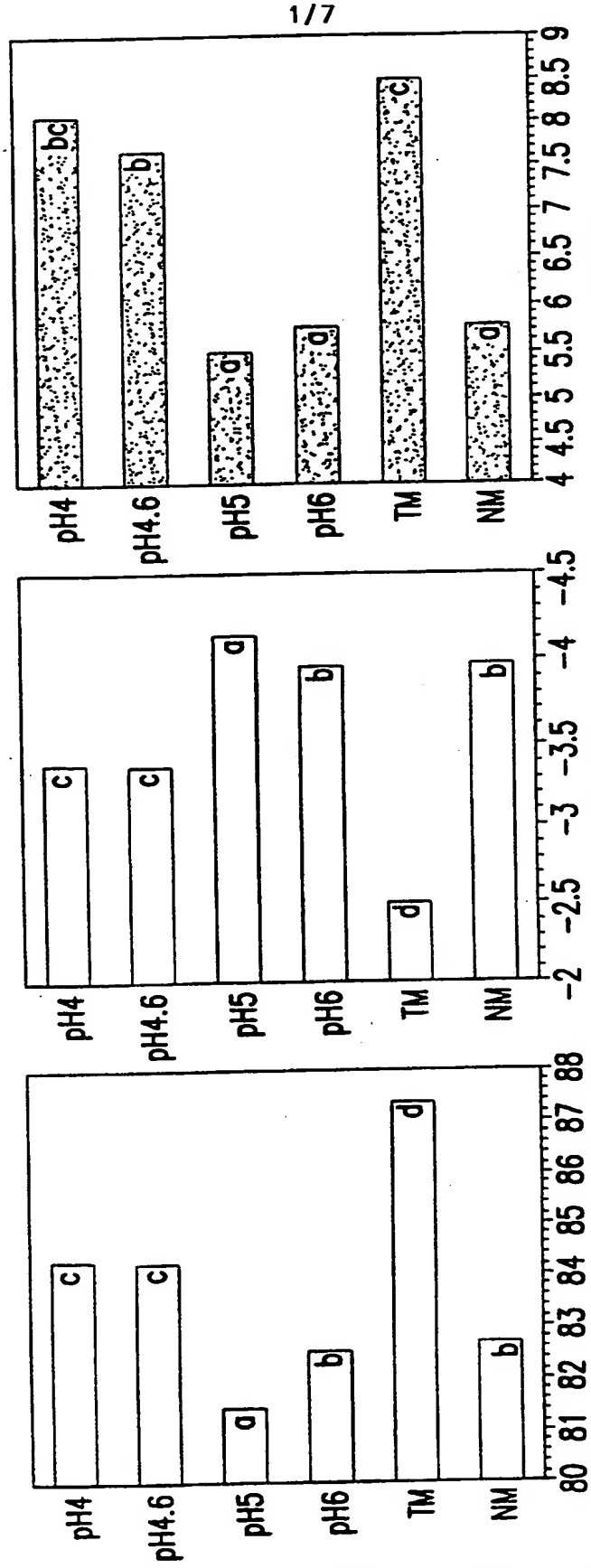


FIG.1C

FIG.1B

FIG.1A

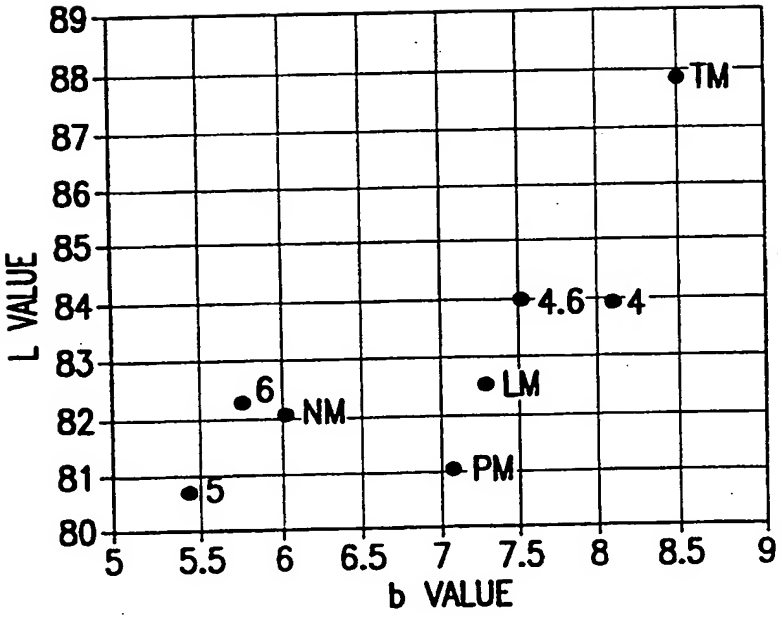

89
88
87
86
85
84
83
82
81
80
L VALUE
5
5.5
6
6.5
7
7.5
8
8.5
9
b VALUE
TM
4.6
4
LM
6
NM
PM
5

FIG.2

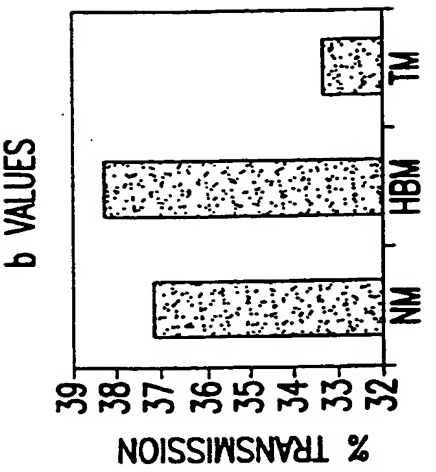
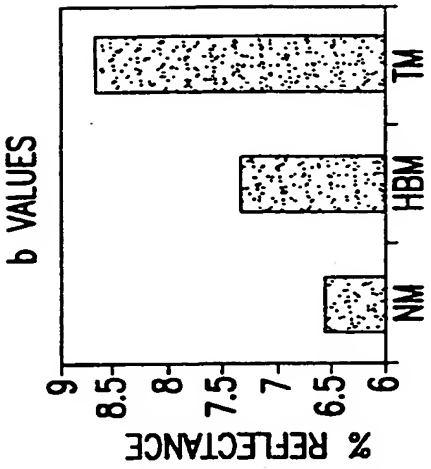


FIG.3C



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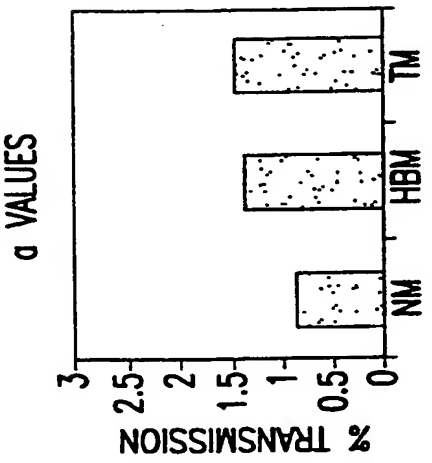


FIG.3B

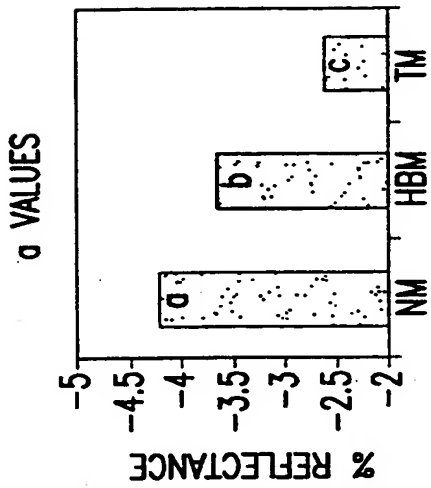


FIG.3E

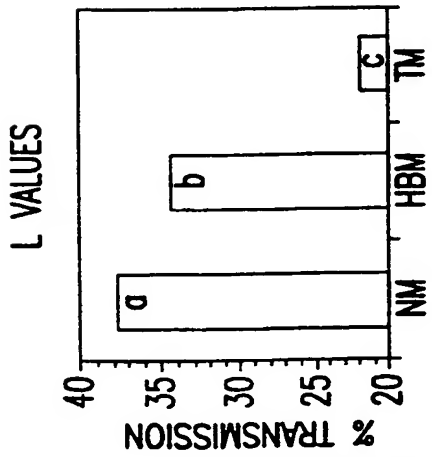
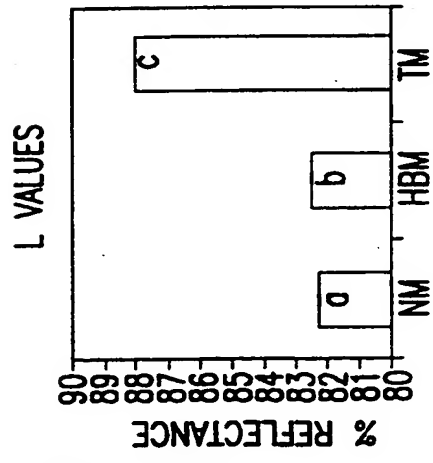


FIG.3A



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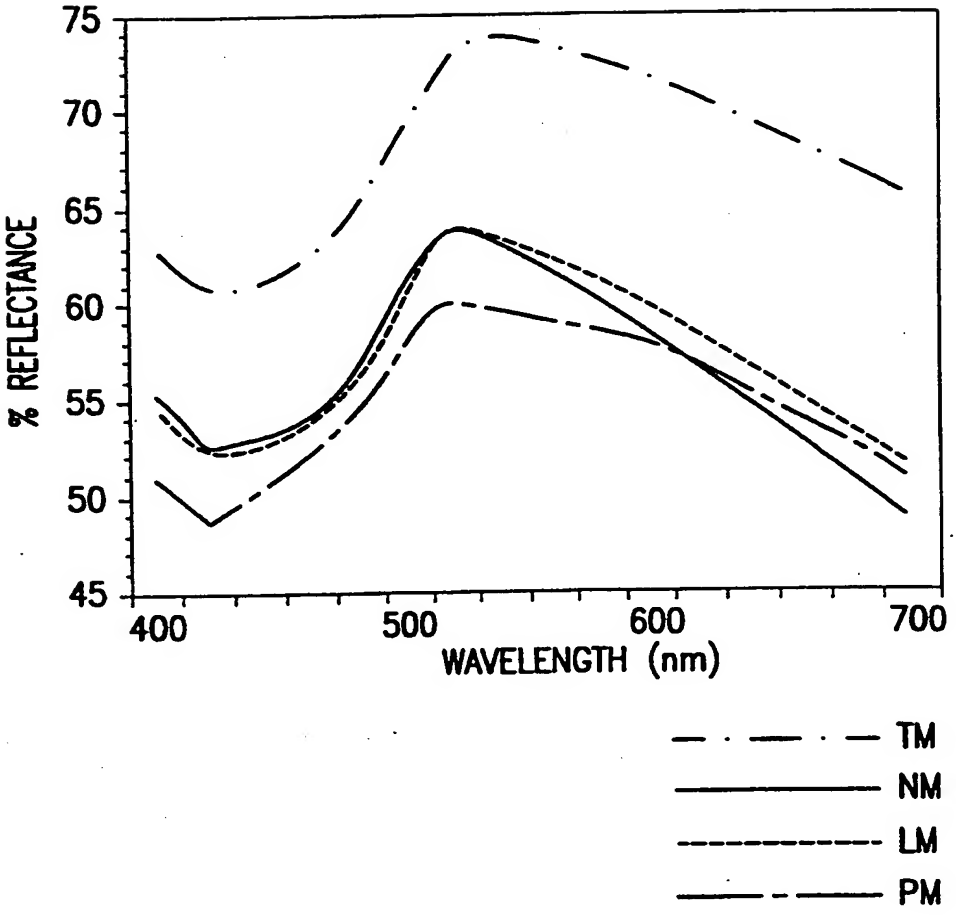
75
70
65
60
55
50
45
% REFLECTANCE
400
500
600
700
WAVELENGTH (nm)
TM
NM
LM
PM

FIG.4

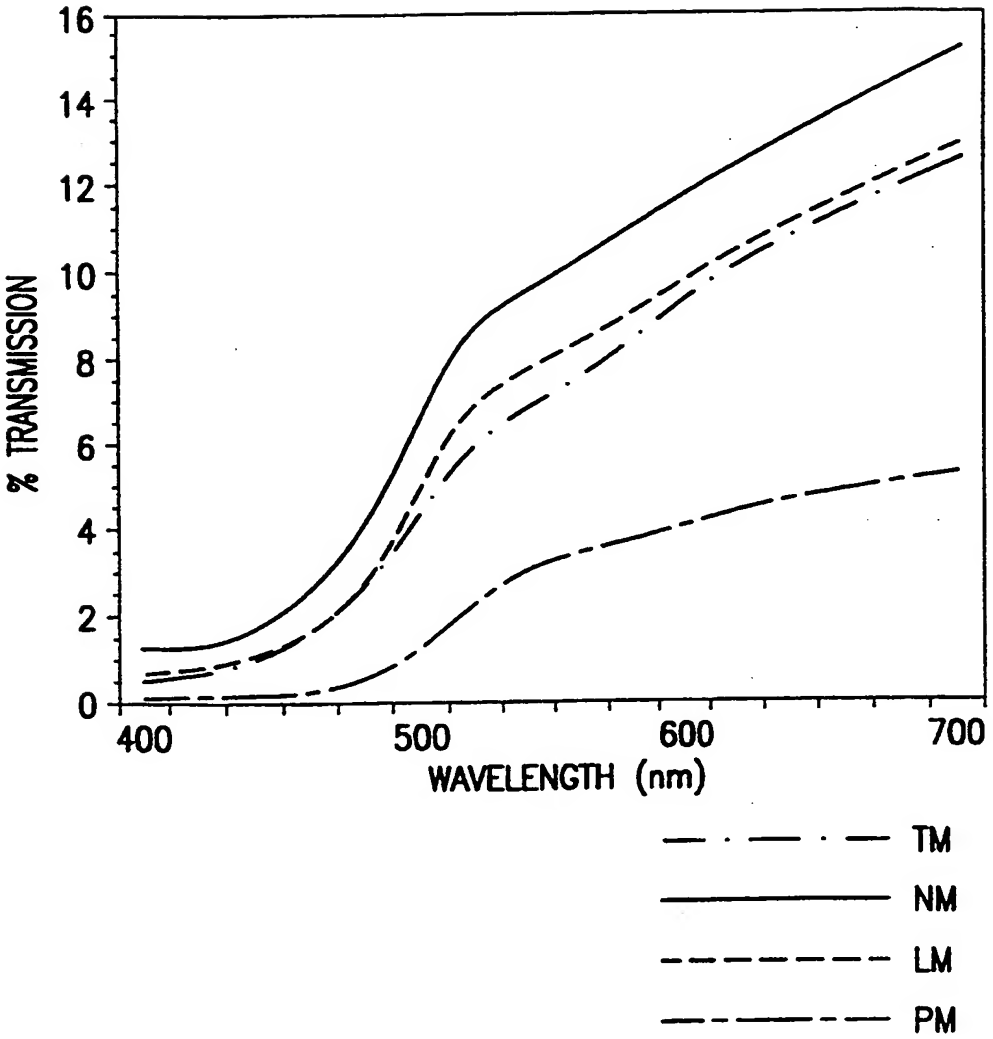
16
14
12
10
8
6
4
2
0
% TRANSMISSION
400
500
600
700
WAVELENGTH (nm)
TM
NM
LM
PM

FIG.5

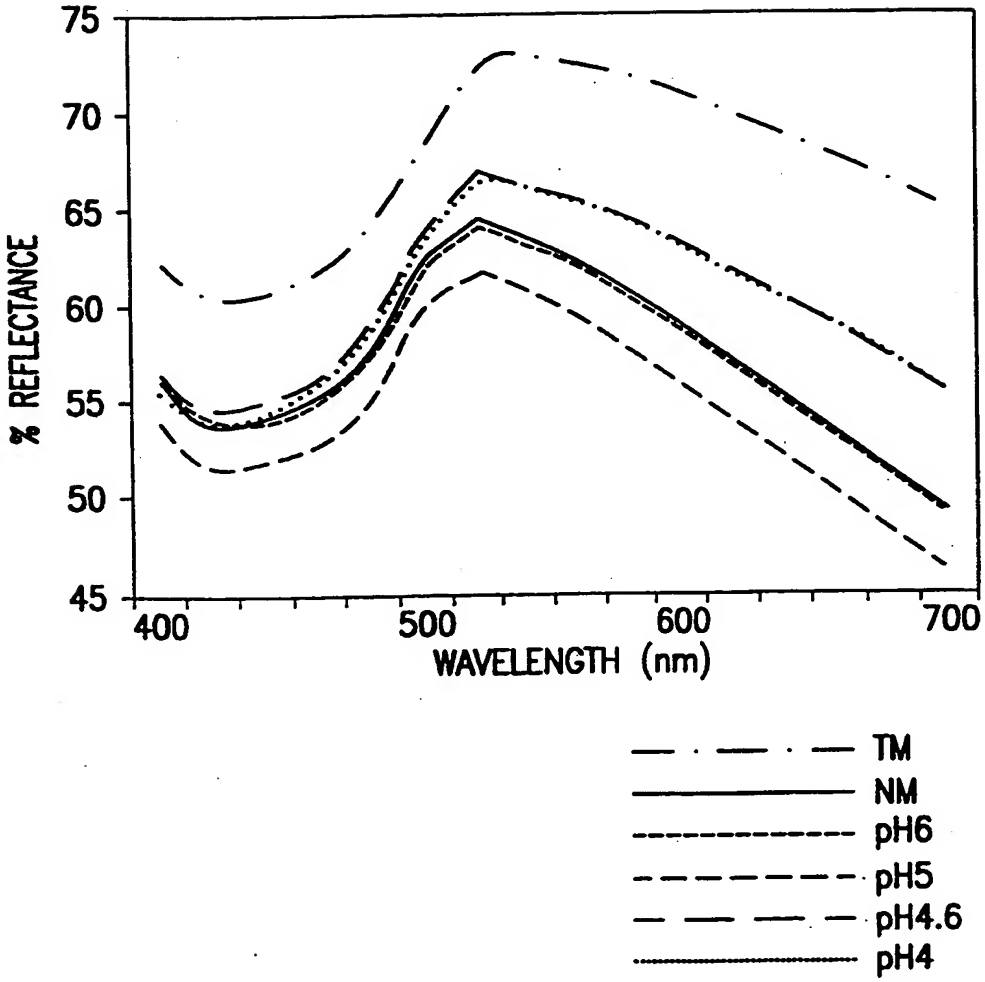
75
70
65
60
55
50
45
% REFLECTANCE
400
500
600
700
WAVELENGTH (nm)
TM
NM
pH6
pH5
pH4.6
pH4

FIG.6

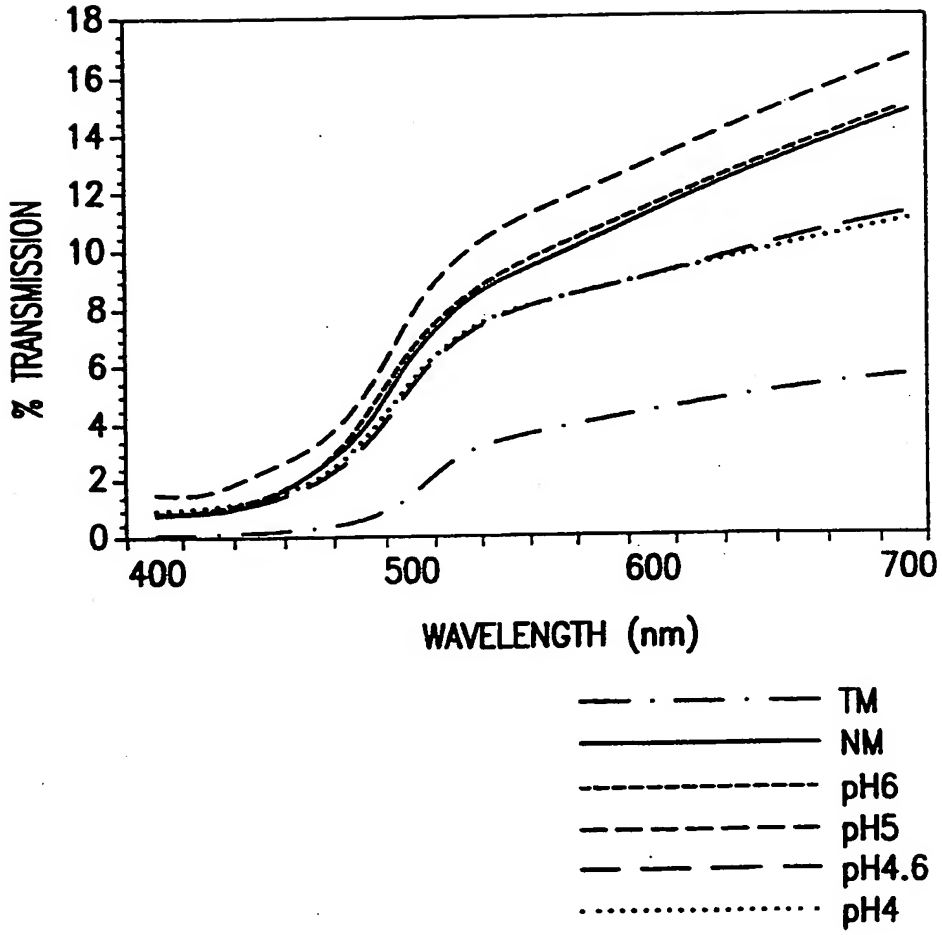
18
16
14
12
10
8
6
4
2
0
% TRANSMISSION
400
500
600
700
WAVELENGTH (nm)
TM
NM
pH6
pH5
pH4.6
pH4

FIG.7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/16870

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A23C 9/12
US CL :426/034

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 426/034, 042, 043, 580, 583

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

NONE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RANKIN ET AL, Color of Nonfat Fluid Milk as Affected by Fermentation, Journal of Food Science, Volume 63, No. 1, January-February 1998, pages 178-180.	1-19

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
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Date of the actual completion of the international search
20 SEPTEMBER 1999

Date of mailing of the international search report
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